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ON THE OCCURRENCE OF A TRYPANOPLASM, PROBABLY *TRYPANOPLASMA BORRELI* LAVERAN ET MESNIL, IN THE BLOOD OF THE COMMON SUCKER, *CATOSTOMUS COMMERSONII*[1]

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In spite of the considerable interest in the distribution of the hemoflagellates, there is no record, so far as the writer is aware, of a trypanoplasm occurring in the New World. The species to be described seems to be identical with *Trypanoplasma borreli* described by Laveran and Mesnil (1902). This species is reported to cause disease and death in fish in captivity, but no case is recorded of a trypanoplasm causing a pathogenic condition in a fish in the wild state, where there is little opportunity for more than a single infection. On this account the facts to be recorded are of special interest.

The sucker in which the trypanoplasm was found was seen in shallow water at a wharf in Go Home Bay, a small bay leading from the Georgian Bay, about twenty miles from Penetanguishene, Ontario. The fish was sluggish and allowed itself to be easily picked up in a dip net. When brought to the laboratory and taken out of the water it died in a few minutes. There were no external lesions and no abnormalities were discovered in a hasty examination of the viscera. The gills were pale and bloodless. Preparations of blood from the heart showed the presence in great abundance, one or two in a single field of the immersion objective, of the trypanoplasm to be described.

As the Biological Station was about to be closed for the season it was possible to obtain only one other sucker, which was caught in a fyke net. This was active and normal in every way. In five fresh preparations of the blood from the gills, the heart, and the liver no hemoflagellates were found after a careful search.

The evidence available in this case is scarcely sufficient to prove the trypanoplasm as a specific cause of the pathogenic condition of the

1. The observations in this paper were made while the writer was curator of the Biological Station of the Canadian government at Go Home Bay, near Penetanguishene, Ontario, in 1913. The courtesy of the directors of the Biological Board of Canada permits their publication here.

EXPLANATION OF PLATE

Trypanoplasma, probably *T. borreli.* All the figures with the exception of Figure 1, which is a free-hand drawing, were drawn with the camera lucida, using a Leitz 2 mm. apochromatic objective and compensating ocular × 18. They have been reproduced at a magnification of 2,600 diameters. Figures 2-6 were drawn from smears fixed with osmic acid vapor, stained with Giemsa's azur-eosin and mounted in neutral Canada balsam.

Fig. 1. Drawn from a living specimen in a fresh preparation of the blood.

Fig. 2. From blood from the kidney.

Fig. 3. From blood from the heart.

Figs. 4 to 6. From blood from the kidney.

fish. The parasite is interesting, however, in that it occurs in a fish in the wild state, where there is little probability of there being more than a single, or at most, a few infections, the parasite being without doubt carried by a leech. There is no record, so far as the writer is aware, of a trypanoplasm causing a pathogenic condition in a fish in the wild state. Such a condition, however, has been recorded for fish in captivity, and if, as the author is inclined to believe, the parasite of the sucker is *T. borreli,* for the same species of parasite. In carp, *Cyprinus carpio L.,* infected with *T. borreli,* Marianne Plehn (1904:175) finds: "Bei stark befallenen Fischen erreicht die Anämie einen ganz extremen Grad; man kann nur wenige Tropfen eines wässerigen, kaum rötlichen Blutes gewinnen; Keimen und innere Organe sind áusserst blass. Andere pathologisch-anatomische Merkmale fehlen. Die Tiere zeigen in der letzten Lebenszeit ausser beschleunigter Atmung und grosser Unlust sich zu bewegen nichts Auffälliges. Sie gehen offenbar an Blutmangel ein, den sie, zwar lange, aber doch nicht dauernd ertragen können. Es ist unzweifelhaft, dass die Krankheit auch im Freien Schaden anrichten wird; die Beobachtungen sind noch zu jungen Datums um allgemeine Angaben über Verbreitung und Bedeutung zu gestatten." Keysselitz (1906) has also described the pathological condition in the carp due to *T. borreli.* Leger (1904:824) describes cases of acute infection of the minnow, *Phoxinus laevis* Agass, with a trypanoplasm as follows: "Des infections aussi intenses amènent chez le poisson une anemie profonde: decoloré et enflé il se tient immobile, refuse toute nourriture et finit par mourir." A case in which a trypanosome may cause a pathogenic condition has been recorded by Doflein. He remarks (1909:398), referring to *Trypanosoma carassii:* "Ich selbst hatte allerdings einmal Gelegenheit, eine sehr ähnliche, vielleicht sogar identische Art im Blut der Schleihe, *Tinca vulgaris,* zu beobachten; die befallenen Schleien waren offenbar krank, sie waren sehr apathisch und waren an die Station zur Untersuchung von Fishkrankheiten in folge eines grossen Sterbens in den betreffenden Weihern eingesandt worden."

The terminology used in this paper is that used by Minchin (1912), with the exception of the term "basal granule," which has been used in place of the term "blepharoplast," as used by that author.

As seen in the living state (Fig. 1), the trypanoplasm has the form typical of the genus; a thick yielding body with two flagella, one of which forms the border of an undulating membrane. The measurements of the body are, length 20-25 μ, thickness 3-4 μ. The anterior flagellum is between one-half and two-thirds as long as the body. The posterior flagellum arises near the anterior flagellum and passes posteriorly, forming the margin of the undulating membrane. It extends

freely for about two-fifths of its length beyond the posterior end of the body. The undulating membrane is comparatively thick and not sharply distinguishable from the edge of the body. The parasites showed an active writhing motion, but little progression. What there was seemed to be with the morphologically posterior end in advance. The protoplasm was finely granular, a few larger highly refractive greenish granules measuring up to 0.4 μ in their longest diameter, being present in the posterior half (Fig. 1). After the preparation had been standing for a little time, sealed with vaselin, two or three large vacuoles were seen in the posterior end of some individuals.

Smears of the blood from the heart and from the kidney were fixed in the vapor of osmic acid, stained with Giemsa's azur-eosin and mounted in Canada balsam neutralized with lithium carbonate. In each of the smears the trypanoplasm was found to be abundant. Although often much distorted, the parasite is found in some parts of the smears remarkably well preserved. It is to be regretted that when the parasite was discovered time did not permit of the making of "wet" smears stained with hematoxylin. The results especially as regards the kinetonucleus are open to criticism on that account.

About fifty of the best-preserved and most clearly showing individuals have been studied in detail with a 2 mm. apochromatic objective and compensating oculars 12 and 18. The parasites show great uniformity in size and general structure. They are nearly always sickle-shaped, the trophonucleus and the undulating membrane being on the convex side. Measurements show little or no difference in size between fresh and preserved individuals.

The protoplasm, as in the fresh preparations, is finely granular and contains a varying number of relatively large, deeply staining granules distributed either mainly in the posterior end (Fig. 3), or irregularly throughout its extent (Figs. 4, 5). It is possible that these larger granules are identical with the greenish granules observed in fresh preparations. Such "chromatoid granules" have been found in different species of trypanoplasms (Leger, 1904; Keysselitz, 1906; Friedrich, 1909; Minchin, 1909). It is doubtful whether these granules are chromidial in nature.

The anterior flagellum arises at the extreme anterior end and on the side of the body on which the kinetonucleus is located. It leaves the body independently of the posterior flagellum (Figs. 4, 5). The posterior flagellum arises very close to the anterior and passes around the blunt anterior end and along the entire length of the body as the margin of the undulating membrane (Figs. 1, 2). It is continued posteriorly as a free flagellum of a length equal to about two-thirds that of the body. The undulating membrane shows in some cases as

a clear unstained area between the posterior flagellum and the granular protoplasm of the body (Figs. 2, 4).

The kinetonucleus (Woodcock, 1909, for the "Geisselkern" of German authors) is situated on the side opposite to the trophonucleus and the undulating membrane and about one-third of the length of the body from the anterior end. It has a clear outline and stains deep purple, in contrast to the more reddish tinge of the trophonucleus. In the individuals which show least distortion it is ovoid, about half again as long as it is wide and shows a distinct membrane. Its size varies between wide limits (Figs. 2-5). Its usual size, however, is 3.5 by 2.4 μ. When not too deeply stained five or six deeply staining bodies can be seen lying immediately under the membrane. In some cases what appears to be two kinetonuclei are present in individuals which show no division of the flagella or trophonucleus (Figs. 3, 5). In such cases each of the two bodies shows a clear contour and is undoubtedly surrounded by a membrane. Each also shows the included stained granules, as in the case of the single kinetonucleus, but the number of granules in each is less than in the single kinetonucleus. The two bodies may be of almost equal size, or one, always the anterior, may be much the smaller (Figs. 3, 5); they may be near together or far apart.

That this dual nature of the kinetonucleus is due to faulty technic seems hardly possible, in view of the fact that the two parts are surrounded by distinct membranes. It may be that it is due to division, the kinetonucleus, in this case, having completely divided before either the flagella or trophonucleus. Against this assumption are the facts: first, that the individuals show no other signs of division, unless, which is doubtful, the presence of two basal granules is to be taken as such; and second, that the two parts of the kinetonucleus are often of very unequal size.

Keysselitz (1906) finds in *Trypanoplasma borreli* Laveran et Mesnil that the "blepharoplast" (kinetonucleus) divides transversely, but says (p. 32): "Ein zeitlich gesetzmässiges Verhalten zwischen der Teilung des Kernes und Blepharoplasten kann ich nicht konstatieren." Friedrich (1909:385) finds that the division of the kinetonucleus is "eine einfache Längsspaltung." His figures 19 and 22 show that it may divide before either the trophonucleus or the flagella. Martin (1910) finds in *Trypanoplasma congeri* Elmhirst and Martin that the divisions of the flagella and the trophonucleus precede that of the kinetonucleus.

The condition found in the blood of *Catostomus commersonii* seems to resemble most closely that described by Keysselitz (1906:25) for *Trypanoplasma borreli* in "geschwächten anämischen Fischen" (see his Figs. 40, 42, 45*i*). Here, however, the kinetonucleus may be divided

into more than two parts. In this connection it will be remembered that the sucker in which the trypanoplasm was found showed an anemic condition similar to that described by Keysselitz. The same author (1906:37 and Fig. 47) finds in a parasite of the stomach and adjacent parts of the alimentary tract, "Bei *Trypanoplasma ventriculi* weist der Blepharoplast sehr häufig eine Sonderung in zwei Stücke auf." Laveran and Mesnil (1902) in their description of *T. borreli* figure two individuals (p. 491, Figs. 13 and 15), each with two kinetonuclei. (Although Laveran and Mesnil considered these bodies to represent the trophonucleus "le noyau" and not the "centrome des Trypanosoma," there is no doubt that they were mistaken.)

Two basal granules (centrioles, blepharoplasts of Minchin, 1912) are usually to be seen where the flagella arise. They stain deeply and are to be distinguished only by their position from the chromatoid granules found in other parts of the protoplasm. Although the ends of the flagella can in certain animals be seen to enter the protoplasm separately (Figs. 4, 5, 6), they cannot be traced to separate granules (Figs. 4, 5).

The trophonucleus is situated about a third of the length of the animal from the anterior and often lies side by side with the kinetonucleus (Figs. 2, 3, 5); in other cases it is slightly behind the kinetonucleus (Figs. 4, 6). Its shape and size resemble that of the kinetonucleus, being however usually slightly smaller. It is ovoid and measures on the average 2 by 3 μ. In many cases it shows a distinct membrane (Figs. 2, 4). In other cases such a membrane was not to be seen, probably on account of poor fixation. It contains a varying number of deeply staining granules. In some cases one of these granules (karyosome?) is larger and centrally located.

So far as the writer is aware the genus Trypanoplasma has been described only from European fishes. The species recorded as occurring in the blood are:

T. abramidis Brumpt 1906.
T. barbi Brumpt 1906.
T. borreli Laveran et Mesnil 1902.
T. cyprini Plehn 1904.
T. guernei Brumpt 1906.
T. gurneyorum Minchin 1909.
T. keysselitzi Minchin 1909.
T. truttae Brumpt 1906.
T. varium Leger 1904.[a]

The species abramidis, barbi, cyprini, guernei, gurneyorum and truttae, as described by their authors, have a rod shaped kinetonucleus and the free portion of the posterior flagellum either half as long

(barbi) or less than half as long as the anterior flagellum; two characters which exclude the Trypanoplasma of the sucker. The latter differs, also, from *T. keysselitzi* which has the two nuclei near together at the anterior end and the kinetonucleus "very elongated". There seems some doubt as to whether *T. varium* is not the same species as *T. borreli*, the chief argument of Leger (1904[a]) being that the two forms show a preference for different hosts.

The trypanoplasm found in the sucker has all the morphological characters described and figured for *T. Borreli* by Laveran and Mesnil (1902), size and shape of the body, position and shape of the nuclei, and length of the flagella. The writer therefore provisionally identifies it with this species.

It is interesting to note that German carp, in which Keysselitz (1906) has studied *T. borreli*, have been introduced into the Canadian lakes and occur near where the sucker was found. *Catostomus commersonii* and *Cyprinus carpio* are closely related fish, being in the same family, Cyprinidae.

It is therefore not improbable that *T. borreli* has been introduced into the Canadian lakes with the German carp.

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